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IS THE PRESSOR EFFECT OF PITUITRIN DUE TO ADRENAL STIMULATION

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OUR first experiments were made upon dogs under ether-urethane anaesthesia. In most cases the experiments were begun under ether, then urethane (two grams per kilo) was immediately introduced into the stomach and the ether gradually discontinued as the urethane became effective. In some instances, however, a slight amount of ether had to be continued throughout the experiment in order to maintain satisfactory anaesthesia. Cannulas were introduced into the carotid artery for the registration of blood pressure and into the femoral vein for the administration of the drug under investigation. The belly was opened in the median line and, while the viscera were protected by warm towels, needles were thrust through the body wall at each side of the hilus of each adrenal gland. By means of these needles double ligatures were drawn through so that they could be pulled tight from outside the body and occlude adrenal circulation. The ligatures were carefully placed to avoid including any considerable number of splanchnic nerve fibres, the blocking of which would introduce an extraneous factor. The ligatures were used double to permit removal with a minimal laceration of tissues; they were looped together and so adjusted that the junction came at the hilus of the gland. Then when either member of either loop was cut, a slight traction on its fellow-member released both loops. The ligatures

having been adjusted, the abdominal cavity was closed and the experiment begun. This technique has previously been used by several investigators.[1]

Blood pressure was recorded by means of an ordinary mercury manometer and float. Having established the normal pressure for a given animal, a standard dose of pituitrin (Parke, Davis and Co.) was introduced into the femoral vein. In order to avoid a diminution of effect when the dose was repeated, small quantities only were used, — from 0.6 to 10 c.c. of a 1:10 solution. It was found, as a matter of fact, that such dosage could be repeated at intervals of 10 to 15 minutes without significant decrease in pressor effects. Having recorded the results following a given standard dose, the adrenals were ligated by traction upon the previously arranged ligatures. After a brief fluctation blood pressure immediately returned to its previous level. Then the same dose was repeated and the effects again observed. When this effect had worn off, the ligatures were released. Again there was a brief fluctation of pressure, which, however, soon returned to normal, permitting another repetition of the standard dose. Allowing for a slight progressive diminution in the sensitiveness to the drug, there was no appreciable difference between the effects when the adrenals were intact and when their circulation was occluded. Our results confirm the observations of Young and Lehmann and of Hoskins and McClure[2] that adrenal ligation in the dog has no immediate influence upon blood pressure.

An obvious source of error in such results is a possible exhaustion of the adrenal glands as a result of the anaesthetic and of the sensory stimulation necessarily involved in the preparation of the animal. In carrying out another research we obtained evidence which indicates that in the dog such exhaustion actually does occur.[3] Cannon and Nice[4] have shown, however, that there is still dischargable epinephrin in the adrenals of cats even after evisceration. We decided, therefore, to repeat the observations on this animal. Three such experiments

[1] Young and Lehmann: The Journal of physiology, 1908, xxxvii, p. liv. Hoskins and McClure: Archives of internal medicine, 1912, x, p. 343.

[2] *Loc. cit.*

[3] Hoskins and McPeek: The Journal of the American Medical Association, 1913, lv, p. 1777.

[4] Cannon and Rice: This Journal, 1913, xxxii, p. 49.

were made, using correspondingly smaller doses, — about 0.5 c.c. Ether alone was used for anaesthesia. The results in each case, however, confirmed our previous findings. One experiment was particularly convincing. The cat was in a late stage of pregnancy and the adrenals therefore supposedly hypertrophied. That they were actually secreting during the experiment was shown by the fact that the blood pressure was lowered during the time they were ligated off and, after a characteristic epinephrin wave, was re-established at the original level after their release. Pituitrin was injected before, during, and after adrenal ligation; the rise in blood pressure was closely similar in each case.

The presence of accessory chromaffin tissue can scarcely be considered a source of error. If the adrenal glands contain by far the greater portion of such tissue and if the removal of these is without effect, the intact supply can safely be ignored.

The foregoing observations are not without interest in their bearing upon the general problem of the interrelations of the endosecretory organs. Our findings have been offered for publication because in the present state of this subject definite negative observations are nearly as much to be welcomed as further positive results. So far as a relationship between the adrenals and the pituitary is concerned, there is to be found in the literature little evidence. The fact that both adrenal and pituitary extracts raise blood pressure and cause hyperglycemia[5] suggests that one organ might stimulate the other. There are on record observations by Hallion and Alquier[6] and by Rénon and Delille[7] that the prolonged use of extracts of the posterior lobe of the pituitary causes a hyperplasia of the adrenal cortex. In our present ignorance of the physiology of the adrenal *cortex*, however, the significance of such observations is obscure. On the whole, it now seems probable that there is no direct dependence of the adrenals upon pituitary functioning.

[5] WEED, CUSHING, and JACOBSON: Johns Hopkins Hospital bulletin, 1913, xxiv, p. 40.

[6] HALLION and ALQUIER: Comptes rendus de la Société de biologie, 1908, lxv, p. 5.

[7] RÉNON and DELILLE: *ibid.*, p. 499.

SUMMARY

1. Intravenous injections of pituitrin in small dosage can be repeated at intervals of ten or fifteen minutes without significant failure of their pressor effect.

2. The adrenal glands of the dog can be ligated off without affecting general blood pressure; in the pregnant cat, however, such ligation has been observed to cause fall of blood pressure with subsequent rise when the ligatures were released.

3. In either animal occlusion of the adrenal circulation does not diminish the pressor effect of a standard dose of pituitrin.

4. There is probably, therefore, no direct dependence of adrenal functioning upon pituitary secretion.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

V. The Influence of Stimulation of the Gastric Mucosa on the Contractions of the Empty Stomach (Hunger Contractions) in Man

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Analysis of the Problem

THE character of the periodic and continuous motor activity of the empty stomach in man has been reported. It has also been shown that the contractions of the empty stomach give rise to the sensation of hunger, or the "hunger pangs" by stimulation of afferent nerves endings in the walls of the stomach, and not by stimulation of nerve endings in the gastric mucosa. The hunger contractions of the stomach are inhibited, reflexly, by all stimuli acting on the end organs of taste and general sensation in the mouth cavity, so that in the case of chewing palatable food when in hunger we have the so-called psychic secretion of gastric juice preceded and paralleled by a psychic inhibition of gastric motility and tonus. The fact that the hunger contractions of the stomach lead to increased excitability of the central nervous system and to vase-motor changes has also been placed on record.[1] In the present paper an attempt is made to determine more specifically the cause of the hunger contractions themselves, so far as this is possible in man. The contractions of the empty stomach may be due to:

(1) **The Condition or the Stimulation of the Gastric Mucosa.** — The absence of food means absence of mechanical stimuli, and cessation or diminution of the secretion of gastric juice, and hence a diminished acidity. Carbon dioxide may be secreted into the empty stomach and may act as the primary stimulus. Carbon dioxide and

[1] Carlson: This journal, 1912–13, xxxi, pp. 151, 175, 212, 318.

other gases may enter the stomach from the intestines, and act as stimuli. Succus entericus, pancreatic juice, and bile may enter the stomach and act as the primary stimulus through alkalinity or by means of specific substances such as the bile acids. The reader will recall that a number of workers maintain that bile facilitates the intestinal movements.

(2) **The Condition of the Blood,** such as the relative concentration of nutrient substances, tissue metabolites, and hormones. It is possible that the neuro-muscular apparatus of the stomach is specially sensitized to slight variations in these substances. While we recognize the condition of the blood as a possible factor, it does not seem a probable one; in the first place, because the composition of the blood is on the whole more constant than the composition of the tissues, and because in young and vigorous individuals the hunger contractions of the stomach begin as soon as the stomach is empty, and while digestion and absorption is still in progress in the intestines, so that there can be no lack of nutrient substances in the blood. In view of the relative constancy of the composition of the blood as shown by all past work on the serum, the existence of a periodic fluctuation in the concentration of any one substance in the blood parallel with the periodicity of the hunger contractions seems improbable.

(3) **The Nervous Impulses Through the Vagi.** — It is well known that the tonus of the stomach depends, in part, on impulses from the vagi, and that the stimulation of the peripheral end of the vagi induces strong contractions in the stomach whether empty or filled with food. It is also known that the stomach is capable of carrying out the *movements of digestion* to a fair degree of efficiency after section of both the vagi and the splanchnic nerves. In other words, the neuro-muscular apparatus of the stomach seems to be primarily automatic, as regards the genesis of the movements of digestion.

The experiment of sectioning the vagi does not prove this point, however. The experiment does prove the *plasticity* of the gastric motor mechanism. One would expect that the extrinsic gastric nerves bear the same relation to the movements of the filled and of the empty stomach. This phase of the problem cannot be studied on man. If it should develop that the periodic hunger contractions

of the empty stomach are caused by periodic discharges through the vagi, the ultimate question of the cause of hunger would again become a problem of physiology of the central nervous system.

(4) **A Primary Automaticity of the Local Neuro-Muscular Mechanism of the Stomach.** — This can be established only by exclusion of the three other possibilities outlined above. A primarily automatic mechanism might still be influenced by the blood, by the extrinsic nerves, and by local reflexes from the gastric mucosa. The periodicity of the automatic activity might be due, not to a parallel periodicity in any essential stimulus, but to some peculiarity in the metabolism of the stomach developed as a special adaptation, similar to periodicities in other organs. The absence of the hunger contractions during digestion, or possibly the modifications of the hunger contractions into the movements of digestion, must, in this case, be due to specific inhibitory or regulatory impulses from the gastric mucosa.

Mr. V. is admirably adapted for determining the relation of stimulation of the gastric mucosa to the hunger movements, as the fistula is large enough to permit the balloon and connecting tube, and a tube for the introduction of liquids and gases, to be placed in the stomach simultaneously. The liquids and gases can be introduced with or without the man's knowledge. Furthermore, the contents of the stomach (fluid and gas) can be withdrawn for analysis at any stage of the hunger movements and without any material disturbance.

Results

(1) **The Action of Water.** — Water, at body temperature or nearly ice cold, inhibits the tonus and the hunger contractions of the stomach. The inhibition following the introduction of a glass of water (100–200 cc.) directly into the stomach lasts on the whole only three to five minutes, and is never followed by any augmentation of the tonus or the hunger contractions. The cold water causes greater inhibition than the water at body temperature. If the water is introduced into the stomach during very intense hunger contractions ("hunger tetanus") there may be no perceptible inhibition. In other words, the degree of inhibition by water in the stomach is inversely proportional to the intensity of the hunger contractions

present at the time the water is introduced. Water, warm or cold, introduced directly into the stomach during a period of relative relaxation and quiescence does not increase tonus or initiate a contraction period. A typical tracing showing this temporary inhibition by water is reproduced in Fig. 1.

The statement that cold water causes on the whole greater inhibition than water at body temperature requires the following qualification. The record of the stomach movements were taken by means of an air-inflated balloon in the stomach cavity. Now, when cold water is introduced the water surrounds the balloon, at least partly, and cools the air in the balloon. This itself will lower the tension somewhat, until the temperature of the air is restored to that of the body by the warming of the water or by the passing of the water into the intestine. I do not think that this is a serious source of error, for this reason. A few experiments were made with water at 50°C. This causes greater inhibition than the water at 38°C. Water at 50°C. will, of course, increase the air tension in the balloon, yet the inhibition of the stomach tonus and movements is sufficiently marked to mask the effect of slight warming of the air.

How does water in the stomach produce this temporary inhibition? It goes without saying that in these experiments the water was not introduced fast enough to cause contractions by distension of the stomach walls, although this occurred unavoidably in a few instances. The only possible ways that water at body temperature can stimulate the nerve endings in the mucosa seem to be (1) by mechanical pressure, or (2) by osmosis. Cessation of the inhibition probably marks the passing of the water out of the stomach into the intestine, or the addition of sufficient salts to prevent stimulation by hypotonicity. The greater inhibitory action by cold water and by water above the body temperature is evidently due to stimulation of the protopathic temperature nerve endings in addition to those acted on by pressure and osmosis.

It is clear that the action of water on the stomach mucosa is in the direction of inhibition of the hunger contraction. How can this be reconciled with the view that a glass of cold water induces or augments hunger? It is to be remembered that in these experiments the water had no chance to act on the nerve endings in the mouth and the œsophagus. The alleged action of a cold drink on hunger

and appetite is probably reflex effects (cold) from the mouth and œsophagus. In the writer's own case a glass of ice water causes increased mucular tonus, sometimes even to the point of shivering and formication. This increased kinestetic sense probably acts in the way of " bahnung " for the hunger sensation, if it is not actually a part of the hunger complex. Cannon and Washburn [2] suggest that the effect of a cold drink on the hunger sensation is due to " the power of cold to induce contraction in smooth muscle." Although their meaning is not clear I take it that they have in mind primarily the contraction of the stomach musculature. This could not come about by the cold acting on the stomach musculature directly. The reflex effects of cold water from the mouth and œsophagus are very complicated as regards the stomach, while cold water acting on the gastric mucosa directly causes inhibition.

(2) **The Action of Acids.** — All acids, or liquids containing acids, including normal human gastric juice, cause inhibition of the movements and the tonus of the empty stomach when introduced directly into the stomach cavity. No acid has been tested in stronger concentration than 0.5 per cent. The duration of the inhibition is on the whole directly proportional to the concentration and the total quantity of acid introduced. 200 c.c. of 0.5 per cent HCl may cause complete inhibition of the contractions and a relaxation of the tonus for 40–60 minutes, while 200 c.c. of 0.25 per cent HCl will usually inhibit for a period of 25–30 minutes only.

This inhibition by acids can be made evident during all stages of activity of the empty stomach. If the acid is introduced during relative quiescence of the stomach the appearance of the next period of hunger contractions is delayed; if introduced during the active contractions these are abolished or depressed.

The duration of the acid inhibition is probably determined by three factors, namely, (1) passing of the acid into the duodenum, (2) fixation and neutralization of the acid of the mucous gastric secretion, (3) neutralization by bile and intestinal juice which at times pass into the stomach through the dilated pylorus.

While it is a striking fact that gastric juice of full normal acidity (0.48–0.53 per cent) and other acid solutions inhibit the hunger contractions, it does not follow that a neutral or alkaline reaction in the

[2] Cannon and Washburn: This journal, 1912, xxix, p. 452.

gastric cavity is a prerequisite for these contractions. During the strong contractions the stomach secretes a juice rich in mucin and combined HCl, but usually containing some free HCl. After the introduction of acids the contractions reappear before all the acid has passed out of the stomach or has been completely neutralized. And in case Mr. V. chews palatable food during a strong hunger period, the hunger contractions reappear before there is complete cessation of the psychic secretion of gastric juice. In other words, the hunger contractions are not inhibited by weak concentrations of acids in the stomach. A neutral or alkaline reaction of the mucosa is not necessary for these contractions.

A typical tracing showing the inhibition of the hunger contractions by V.'s own gastric juice is reproduced in Fig. 2. When V. chews palatable food during a hunger period two inhibitory factors come into play, namely the reflex inhibition from the mouth, and the acid inhibition from the stomach. If the food is sufficiently palatable and the mastication is continued long enough the inhibition produced reflexly from the mouth fuses with the acid inhibition from the stomach. If the food is not especially palatable or the mastication period brief, the contractions may resume on cessation of the chewing and then again be inhibited for a time during the period of most rapid secretion of the gastric juice.

The degree of inhibition produced by normal gastric juice is the same as that caused by an equal quantity of hydrochloric acid of a concentration equal to the free acidity of the gastric juice. It would thus seem that the hydrochloric acid in the gastric juice constitutes the stimulus that leads to the inhibition.

This acid inhibition of the hunger contractions is of peculiar interest in connection with the neuro-muscular mechanisms of these hunger movements and the gastric movements in normal digestion. The movements of the stomach in digestion are not inhibited by acids in the stomach, that is, at least not by acids in concentrations equal to that of the gastric juice. The fact that the intensity of movements of the antrum increases as the gastric digestion advances may even indicate that a certain degree of free acidity facilitates the movements of digestion. At first it occurred to me that since acid in the stomach inhibits the *hunger contractions*, but not the *digestion contractions*, the mechanisms involved in these two types of gastric

activity are different, at least as regards the character of the afferent impulses from the gastric mucosa. But on further reflection it became apparent that this is not necessarily the case. For the digestive movements involve primarily the pyloric end, while the hunger movements (as studied by our method) involve the fundus of the stomach. It is possible that acid stimulation of the nerve endings in the gastric mucosa leads, reflexly, to inhibition of the fundus, and peristalsis of the pyloric region of the stomach. This hypothesis is, of course, capable of experimental verification or refutation.

(3) **The Action of Alkalies.** — The tests were made with sodium carbonate in concentrations varying from 0.2–1.0 per cent, and in varying quantities. In concentrations of 0.2 per cent or less the sodium carbonate solution appears to have the same influence on the hunger contractions as equal quantities of water, that is a slight temporary inhibition. This inhibition is evidently due, not to the *alkalinity* but to the *bulk* of the solution. In concentrations from 0.2 per cent to 1.0 per cent the degree of inhibition produced is on the whole directly proportional to the concentration and the quantity of the solution put into the stomach. 200 c.c. of one per cent sodium carbonate causes about the same degree of inhibition as 200 c.c. one-half per cent hydrochloric acid. It is thus clear that alkalinity has the same effect as acidity, only to a less degree; both acids and alkalies causing inhibition without any after effect of the nature of augmentation.

The fact that 0.2 per cent sodium carbonate has no more effect on the hunger movements than equal quantities of water seems to show that a slight alkalinity of the gastric mucosa is compatible with the hunger contractions of the empty stomach. It makes it also evident that the entrance of bile or intestinal juice into the stomach will have little or no effect on these movements, while any concentration that influences these movements produce inhibition.

(4) **The Action of Local Anaesthetics.** — Solutions of some local anaesthetics were tested with the view of determining whether the sensory nerves in the gastric mucosa plays only an inhibitory rôle in the processes of gastric hunger contractions. Phenol, chloreton, orthoform, quinine-urea-hydrochloride, and adrenalin chloride were used in quantities and concentrations compatible with *absolute*

safety to Mr. V. It was not considered advisable to use cocaine. The solutions of the drugs were introduced in quantities of 100 and 200 c.c.

In the concentrations employed no specific action of any of the above substances could be demonstrated. For example, 100 c.c. of phenol (dilution 1–10,000) has the same effect as 100 c.c. of water, that is, a slight temporary inhibition. The same applies to the other drugs. No appreciable anaesthesia of the gastric mucosa was produced by any of the drugs. It seems probable that the solutions of these drugs pass out of the stomach just as rapidly as equal quantities of water, and hence do not remain long enough in the stomach to produce local anaesthesia. Because of the danger attending the use of local anaesthetics in strong concentrations, further work on V. was deferred until complete orientation was at hand from work on dogs. It seemed, however, that adrenalin chloride introduced into the stomach even in considerable quantities could not be particularly injurious. But even in large quantities (100 c.c. of a dilution of 1–10,000) the adrenalin acting in the gastric cavity has no other effect on the hunger movements than equal quantities of water.

(5) **The Action of Alcoholic Beverages.** — Tests were made with sour and sweet wines, beer, brandy, and pure alcohol. The taking of alcoholic beverages with the meals is a habit with many people. It is claimed by many people that a glass of wine, beer, or some mixture of alcohol taken before meals increases the appetite (and possibly the hunger). The writer is neither a total abstainer nor a habitual user of alcoholic beverages. But it is his experience that a glass of beer or brandy taken at meal time *seems* to awaken or increase appetite. This effect is rather immediate and therefore not due to the absorption of the alcohol. Powlow has recorded an instance from his own experience where a drink of wine seemed to initiate the sensation of hunger (?) the very minute the wine reached the stomach. From enquiries as extensive as opportunities have permitted, I am inclined to believe that this apparent augmentation of the appetite by alcoholic beverages is rather a common experience. In view of this fact I expected to find that these alcoholic beverages increased the tonus and the contractions of the empty stomach, since it is the tonus and the contractions of the empty stomach that give rise to the hunger sensation. To my surprise the results proved to

be the very opposite. *Wine, beer, brandy, and pure alcohol introduced directly into the stomach inhibit the hunger contractions and the tonus of the empty stomach instead of increasing them.* This is true whether these fluids are cold or at body temperature. If these alcoholic beverages are greatly diluted with water, a degree of dilution can be reached which has the same action on the stomach as equal quantities of water, although the specific beverage is readily detected when the mixture is placed in the mouth. In no instance have I been able to make out any undoubted augmentation of the stomach tonus and hunger contractions after the inhibition period. In other words, alcoholic beverages when introduced directly into the empty stomach in quantities and concentrations that directly affect the tonus and the contractions of the stomach cause inhibition, and inhibition *only*.

The pure alcohol was never used in stronger concentrations than 10 per cent. The brandy was usually diluted one half with water, while the beer and wines were put into the stomach undiluted.

We have seen that acids in the stomach cause inhibition of the hunger contractions. Pure alcohol also causes inhibition. It is therefore evident that the alcohol and acids are primarily responsible for the inhibition following the introduction of alcoholic beverages into the empty stomach. But for the sake of brevity we may designate it "the alcohol inhibition."

The duration of the alcohol inhibition varies directly with the quantity and concentration of the beverage introduced in the stomach. Thus 50–100 c.c. of 10 per cent alcohol may inhibit the hunger contractions for one to two hours; or if introduced during a period of relative quiescence it delays correspondingly the onset of the next hunger period. 200 c.c. of beer causes inhibition for 30–60 minutes. The sour wines on the whole cause greater inhibition than the sweet wines, probably through their acids. A typical record of the alcohol brandy inhibition of the hunger contractions is reproduced in Fig. 3. This tracing is from a series of experiments on the author himself.

It must be stated that these alcoholic beverages were put into the stomach of Mr. V. with his consent and without any protest, resentment, fear, or disgust on his part, which might account for the stomach inhibition. Mr. V. takes wine and beer occasionally. At times I had him bring his own choice of wine and beer, and occasionally I had him, himself, introduce into the stomach the desired

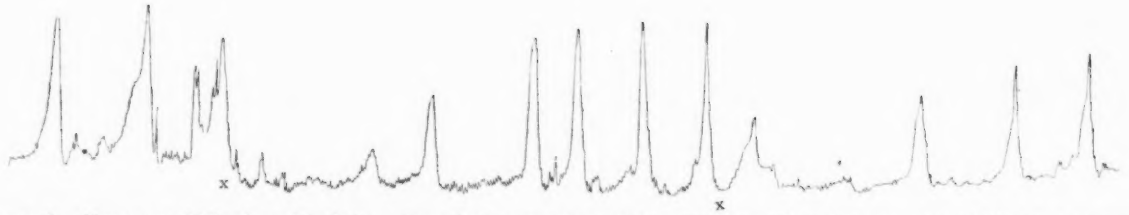

FIGURE 1. About two thirds the original size. Record of contractions of the empty stomach of Mr. V. At × 100 c.c. cold water introduced directly into the stomach. Showing the temporary inhibition.[3]

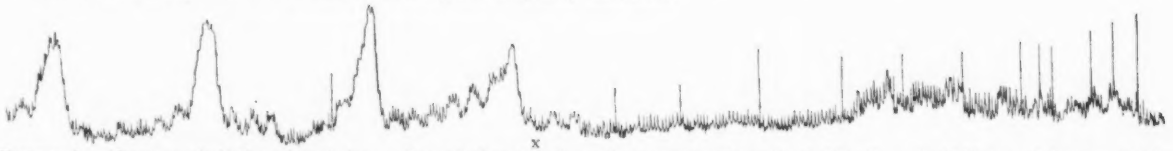

FIGURE 2. About one half the original size. Record of contractions of the empty stomach of Mr. V. At × 25 c.c. of human gastric juice (V.'s own gastric juice, psychic secretion, secured two hours previously) introduced into the stomach. Showing the acid inhibition.

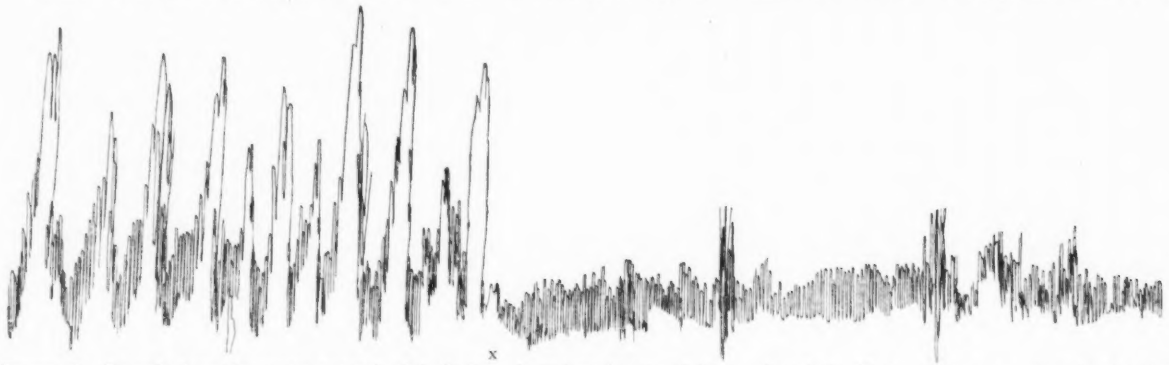

FIGURE 3. Records from the empty stomach of A. J. C. At × introduction of 15 c.c. brandy in 25 c.c. warm water directly into the stomach. Showing the alcohol inhibition of the hunger contractions.

[3] All the tracings reproduced in this paper were taken by means of a bromoform manometer.

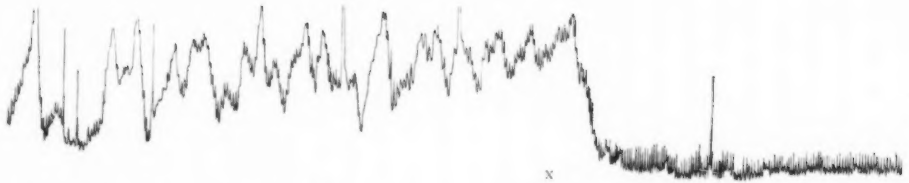

FIGURE 4. One half the original size. Record from the empty stomach of Mr. V. during part of a period of strong hunger movements. At ⊤ 200 c.c. of beer introduced directly into the stomach. Showing immediate and long lasting inhibition of the hunger contractions.

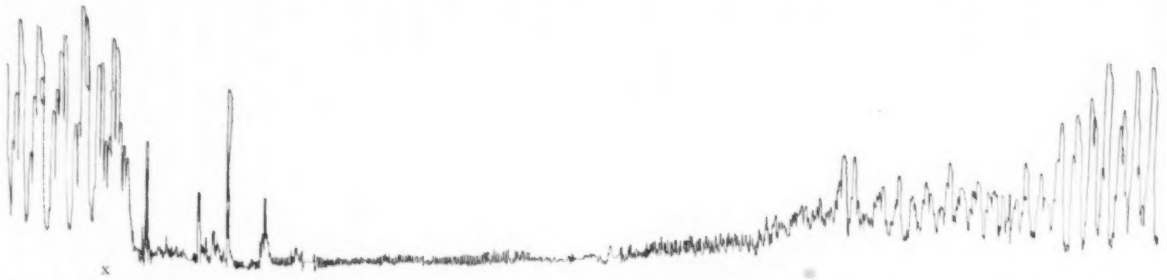

FIGURE 5. About one half the original size. Record from the empty stomach of dog with gastric fistula. At × 25 c.c. of 0.5 per cent HC_l (warm) introduced directly into stomach. Showing the prolonged acid inhibition, with gradual recovery. Total time of the part of the tracing reproduced, 30 minutes.

quantities. The effect on the hunger contractions was always the same. I am therefore confident that we are here dealing with a characteristic alcohol and acid inhibition, and not with a masked "psychic" inhibition.

How are these results to be harmonized with the seeming stimulation of the appetite by alcoholic beverages taken by the mouth? In the first place the local inhibitory action of alcoholic beverages in the gastric cavity is so marked and so invariable, that I feel confident that this is always the gastric effect of these beverages, whether taken normally by the mouth, or introduced into the empty stomach without coming in contact with the mouth and œsophagus. *Alcoholic beverages can therefore not initiate or increase hunger*, since hunger is caused by the stomach contractions, and these are inhibited by the alcohol. Since most alcoholic beverages stimulate the end organs of taste and smell as well as those of general sensibility in the mouth cavity and in the œsophagus it is possible that this stimulation in some way augments or initiates *appetite* for food. If this is the case we have the singular condition of alcoholic beverages augmenting appetite and inhibiting hunger at the same time. There can be little doubt that cerebral states as modified by training and habit are also a factor in this apparent action of alcoholic beverages on appetite. It is certain that the individual's first taste of alcohol, beer, or sour wines does not focus his attention on food and eating.

If alcoholic beverages in the stomach caused as marked inhibition of the stomach movements in digestion as they do in the case of the stomach movements in hunger even moderate drinking with meals would lead at once to acute indigestion. As this is not the case, it is evident that alcoholic beverages affect the mechanisms of these two types of movements differently.

(6) **The Action of Carbon Dioxide and Air.** — The action of carbon dioxide in the cavity of the empty stomach was studied in two ways: (1) by introduction of water charged with CO_2, (2) by introduction of CO_2 gas. It is well known that an excess of carbon dioxide in the blood of the abdominal vessels initiates and augments the tonus and movements of the digestive tract. An excess of CO_2 is sometimes found in the gaseous contents of the empty or partly filled stomach. It is known, furthermore, that carbon dioxide in sufficient concentration acts as a powerful stimulus to the nerve endings in

such membranes as those of the mouth and nose, and of the cornea and conjunctiva.

Carbon dioxide in the cavity of the empty stomach was at first considered a possible stimulus to the gastric hunger contractions, but this hypothesis proved entirely erroneous. In so far as the carbon dioxide in the stomach cavity affects the hunger movements the influence is in the direction of inhibition.

Water saturated with CO_2 under pressure has practically no more effect than similar quantities of pure water. It produces the same degree of temporary inhibition without any after effect in the way of augmentation. As such carbonated water stimulates the nerve endings in the mouth in the characteristic way, it follows that the nerve endings in the stomach are less affected by CO_2 than are the nerve endings in the mouth.

When the CO_2 is forced into the stomach in the form of gas and under pressure, the results are complicated by the mechanical action of the gas in forcibly distending the walls of the stomach and raising the intragastric pressure, and hence increasing the pressure in the balloon in the fundus. A sudden and forcible distension of the stomach no matter how produced leads to a few strong contractions. This factor can be fairly well controlled by introducing the gas slowly. When this precaution is taken the empty stomach can be considerably distended with CO_2 gas, without any marked effect either on the tonus or on the hunger contractions. But the chemical effect of CO_2 so far as it is demonstrable at all, is in the direction of inhibition.

It will undoubtedly occur to the reader that this slight inhibition by the CO_2 may be an instance of "psychic" inhibition from the distress of an overdistended stomach. This possibility has been guarded against. In the first place the stomach was not distended to the point of painfulness by the carbon dioxide. Furthermore, the stomach cavity was irrigated, so to speak, with the gas without raising the intragastric pressure perceptibly, by introducing the inlet tube to the cardiac end and allowing the gas to escape freely by the fistula. The reader will recall that the œsophagus is completely closed, so that the gas cannot escape by way of the mouth. Under these conditions the same slight inhibitory effects were recorded without signs of primary or secondary augmentation. It is thus clear that in so far as carbon dioxide in the gastric cavity affects the gastric

tonus and hunger contractions at all, the action is in the direction of inhibition. This is probably due to the acid stimulation of the nerve endings in the mucosa.

The introduction of air into the empty stomach has no effect whatever on the tonus and the hunger contractions, provided the stomach is not overdistended by the air, or the air introduced rapidly and under such pressure as to cause sudden and forcible distension of the stomach walls. This leads to a few contractions. But the same thing is produced by sudden inflation of the balloon in the fundus. It is therefore purely mechanical. Oxygen in greater concentrations than that of the air has not been tried. But it is evident that the 20 per cent oxygen of the air acts neither favorably nor unfavorably on the hunger movements.

(7) **Confirmatory Observations on Dogs.** — The results of the foregoing observations on Mr. V. are new, and, to the writer's mind at least, unexpected, on the basis of what is known of the motor activities of the stomach. That the same conditions which favor or permit the gastric movements of digestion should invariably inhibit the gastric hunger movements could not have been predicted. The fact that any and all kinds of stimulation of the nerve endings in the gastric mucosa cause inhibition, and only inhibition, of the gastric hunger movements is not to be accepted without rigid scrutiny of the evidence. The observations on Mr. V. were completed in August and September, 1912. They were repeated and confirmed, point for point, in April and May of this year. I am satisfied as to the facts. Is there any error in the interpretation? One possible error in the interpretation of fundamental importance has been referred to once or twice in the previous discussion. It is of sufficient importance to be taken up now, and disposed of as best we may. The fact that nothing but inhibition is produced by substances acting on the gastric mucosa, suggests that this may be in every case a " psychic " inhibition masking any weak action that may be of a positive or augmentation type. The very consciousness of Mr. V. that these substances were introduced into the stomach for experimental purposes might be the primary element in this possible psychic inhibition. That cerebral states may inhibit the gastric hunger movements is certain, from results both on Mr. V. and on dogs. In one instance when preparing to introduce 200 c.c. of 0.5 per cent acidic acid into the

stomach in the midst of the period of powerful hunger contractions Mr. V. somehow thought that I intended to introduce that much concentrated acid (or vinegar). As I was going about with the preparation I noticed that the stomach contractions suddenly became very feeble. Mr. V. looked worried. I inquired if he did not feel right, and he asked if I intended to put all that vinegar into the stomach. "It will surely hurt me," he said. To assure him, I drank half of the acid myself, and then asked him to take a mouthful of it. Then he laughed and said, "Oh, I thought it was pure vinegar." In two minutes after the mental stress and anxiety was over the hunger concentrations returned to their normal rate and amplitude.

The following facts speak against the possibility of the results being due to psychic inhibition.

(1) There was no evidence that Mr. V. was in any way afraid, displeased, disgusted, or impatient with the experiments. This applies particularly to the repetition of the tests in April and May this year. Mr. V. is now usually much interested in all the tests, and he has full confidence in the writer.

(2) The direct proportion between the quantity and concentration of the substance introduced into the stomach and the degree of inhibition produced is contrary to the hypothesis of a psychic inhibition. The displeasure or disgust ought to have been practically the same on introduction of 0.1 per cent and of 0.5 per cent HCl, of 1 per cent and 0.10 per cent alcohol, as in most cases Mr. V. did not know the strength of the material used, and he did not care, being satisfied with my assurance that it was harmless.

(3) In many cases I purposely deceived him as to the nature of the material, exchanging water for acids and *vice versa*. The stomach reaction was invariably in accordance with the substance actually introduced.

Hence I feel fairly satisfied even on the basis of the tests on Mr. V. that psychic inhibition plays no rôle in these results. But to meet the possibility once and for all, I have now repeated and confirmed all of the above tests on dogs.[4] The parallel on the two series on man and dog is complete. The details of these results on dogs will

[4] A series of experiments on myself also confirm the general results as stated above.

be reported later. But one typical tracing may be submitted now (Fig. 5). Well, may not psychic inhibition play a rôle in the tests on dogs? It does not, and for the following reasons:

(1) The dogs could not have known either the difference between the substances introduced into the stomach or the different concentrations of the same substance.

(2) Tests were made during sleep and without the animal waking up. The results were the same.

(3) Psychic inhibition of the gastric hunger movements in dogs is invariably of much shorter duration than the inhibition caused by acids, alkalies, and alcoholic beverages.

It is therefore clear that results on Mr. V. reported above are fundamental facts in the physiology of the stomach and not primarily dependent on afferent impulses that enter consciousness.

(8) **The Influence of the Inhibitions from the Gastric Mucosa on the Fundamental Rhythm of the Gastric Hunger Contractions.** — During the progress of this work it soon became apparent that these temporary inhibitions described above do not cut short a hunger period, but simply delay its culmination. The contractions that appear as the inhibition ceases are the continuation of the period temporarily checked by the inhibition. They are not the beginning of a new period. This is particularly true when the inhibitions are induced during the first half or two thirds of the hunger period. When the tetanus stage of the hunger period is reached a stimulation of the gastric mucosa sufficiently strong to cause prompt cessation of the contractions seems to actually terminate the period, for when the contractions reappear they are not the incomplete tetanus or strong and rapid contractions of the culmination of the period, but the feeble and slow movements characteristic of the beginning of a period. By careful adjustment of the quantity and strength of the material introduced into the stomach during the first part of the hunger period and by renewing the inhibition on reappearance of the rhythm it is possible to lengthen a 30–40 min. period into a 90–120 min. period. In other words, the motor mechanisms of the hunger contractions may be compared to the spring of a watch. When the spring is wound up it will run the watch for a certain number of hours, and it makes no difference whether or not these hours are consecutive.

It seems to me that this fact has an important bearing on the

question of the primary stimulus to the hunger movements. It seems to point to a primary automatism, peripheral or central, or both, relatively independent of the condition of the blood as well as of afferent nervous impulses. The fact speaks particularly strongly against the hypothesis that the primary stimulus is to be sought in the condition of the blood. For example, if the primary stimulus is in some condition of the blood, this condition must be present and to a gradually increasing degree from 12:30 to 1 p.m. to parallel a hunger period beginning at 12:30 p.m. and ending at 1 p.m. And this condition of the blood must be absent from 1 p.m. to 1:45 p.m. as the stomach is relatively quiescent during that time. The hypothesis seems to be rendered untenable by the fact that by manipulations which do not, at least in some cases, involve any change in this hypothetical condition of the blood the culmination of the hunger period may be delayed till 1:30 or 1:45 p.m., so that the strongest hunger contractions fall in the time when the blood does not stimulate the gastric mechanism in a way to cause the hunger movements.

(9) **Discussion of the Results.** — I do not see how the further analysis of the mechanisms of these inhibitions is possible by experiments on man. Whether the reflex is, in whole or in part, through the vagi and the splanchnic nerves or local through the mesenteric plexus can only be determined by lesion of the extrinsic nerves. This phase of the work is not yet completed.

But what is the significance of this inhibition in the normal work of the stomach? The inhibition of the hunger contractions by mechanical and chemical stimulation of the gastric mucosa prevents the appearance of these contractions during the period of gastric digestion. This negative control of the hunger movements from the stomach cavity is obviously a useful coördination. The primary or actual stimulus to the hunger contractions is therefore to be sought in the vagus tonus, in some condition of the blood, or in a primary automatism of the gastric neuro-muscular mechanism. I have some evidence that the latter is the essential factor and that extrinsic nerves and the condition of the blood only modify the primary automatism. If this is the case the hunger contractions ought to appear as soon as the stomach is empty of food or other substances capable of stimulating the nerve endings in the mucosa. We would also

expect these contractions to be more or less continuous as long as the stomach is empty, at least in young and vigorous individuals, and when the condition of the individual as a whole does not lead to increased activity of the extrinsic inhibitory nerves (splanchnics). On this hypothesis the gradual tonus contraction of the gastric fundus *pari passu* with the progress of the gastric digestion represents the algebraic sum of the inherent automatism and the inhibitory effects from the gastric cavity. A gradual fatigue of the inhibitory mechanisms is probably also a factor, as I have abundant evidence (man and dog) of such "escape" of the stomach from inhibitory nervous processes.

We should probably look for the closest parallelism between the gastric hunger contractions and the absence of stimulation of the gastric mucosa in infants and young children, that is, before cerebral (and possibly gastric) habits relative to feeding have been established. During the last five months I have made a close study of a healthy (bottle-fed) infant touching this point. It is well known that, other things being equal, the more food put into the stomach the longer time required for the completion of gastric digestion. If this infant (now five months old) is given only four ounces of food he calls for more after about two hours. If he is given seven to eight ounces of the same food the call for more food is delayed for three to four hours. If he is given five ounces of the food at 6 p.m. he nearly always wakes up and calls for more at 12–1 o'clock; while if he is given as much food as he will take ($7\frac{1}{2}$ to $8\frac{1}{2}$ ounces) at 6 p.m. he rarely wakes up and calls for food until 3–5 o'clock the following morning. There is evidently a close parallel between the time of the emptying of the stomach and the appearance of the hunger contractions. The more frequent calls for food during the day are obviously due to the fact that the gastric hunger contractions must reach a certain degree of intensity before they cause the soundly sleeping infant to wake up. This is certainly true in the case of dogs. A dog may sleep on peacefully and quietly during gastric hunger contractions of moderate intensity. When these contractions become very intense the dog moves or moans in his sleep and sometimes wakes up.

While I have made no observations on the action of acids, alkalies and alcoholic beverages on the gastric movements of digestion, it is quite clear that these substances do not inhibit these movements

at least to the extent that they inhibit the hunger contractions. The movements of digestion are primarily concerned with the pyloric region, while the hunger contractions involve the cardiac and fundus region. Either these two regions of the stomach react differently to local stimulation of the gastric mucosa, or else the nervous mechanisms concerned in the hunger contractions and the digestion contractions are to a certain extent different.

In view of the fact that acids as well as normal gastric juice inhibit the gastric hunger contractions I expect that persons having gastric hypersecretion or actual hyperchlorhydria experience little or no true hunger sensations or pangs of gastric origin. At the same time we must consider in cases of prolonged hypersecretion, the possibility of a readjustment of such a character that the acid stimulation of the mucosa causes less inhibition than is the case in the normal stomach. The degree of plasticity inherent in these gastric mechanisms is virtually unknown.

RAPID METHOD OF PREPARING THROMBIN

By W. H. HOWELL

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A FEW years ago the author described a method of preparing pure or approximately pure thrombin. Beginning with a solution of fresh fibrin in strong solutions of sodium chloride the other proteins were removed gradually by repeated shaking of the solution with chloroform. The method is effective, but it is very tedious, requiring usually two months or more when the solution is shaken twice a day. I have modified the method recently so that an equally good, in some respects a better preparation of thrombin may be obtained in three or four days. This method, in detail, is as follows. A large amount of (pig's) fibrin is obtained from the butcher and is washed thoroughly in running water until the hemoglobin is removed. The washing must be done by hand, picking apart the strands that are deeply colored. When sufficiently washed the mass of fibrin is squeezed free from water and is then minced and well covered with an 8 per cent solution of sodium chloride. The preparation is allowed to stand for forty-eight to seventy-two hours in the cold and is then filtered. The filtrate contains thrombin together with other proteins. The filtrate is treated with an equal volume of acetone. A bulky precipitate is thrown down including the thrombin, while a large amount of the other proteins remains in solution. The precipitate is filtered off rapidly through a number of small filters, each containing from 25 to 50 c.c. As soon as the filtrate has run through, each filter is opened upon a pad of filter paper and the precipitate is spread as thin as possible by means of a spatula. Each filter paper, then, with its layer of precipitate is dried rapidly in a current of cold air.

For this purpose I make use of a box with wire trays and an electric fan. When perfectly dry the portion of the filter paper holding the dried precipitate is cut up with scissors into a bulk of water, using an amount of water equal to about two-thirds of the original saline

filtrate. After standing, with occasional stirring, for one-half hour, this solution is filtered. The filtrate contains the thrombin with traces of salt and of a protein coagulable on heating. To remove this latter protein the solution may be shaken with chloroform (10 or 15 c.c. to 100 c.c. of solution). After shaking, the chloroform settles, on standing, as a thick emulsion. This is filtered off and a specimen of the filtrate is tested by boiling. If the specimen shows any opalescence it indicates the presence still of some coagulable protein, and the process of shaking with chloroform must be repeated once or twice until a specimen of the filtrate remains entirely clear on heating. Care must be taken, however, not to continue shaking with the chloroform after the removal of the coagulable protein, as the thrombin also will come down eventually with the chloroform. This result seems to happen much more easily in these solutions practically free from salt than in the strong salt solutions of my first method. To preserve the thrombin finally obtained my method is to evaporate it to dryness in watch crystals, 2 c.c. to a crystal, using the current of air from an electric fan. When thus evaporated the thrombin shows a characteristic snow-flake crystallization together with a few crystals of sodium chloride. The dried thrombin may be kept indefinitely in a desiccator. It is easily and completely soluble in water and shows the reactions described in my previous paper.

To test the action of thrombin it is very convenient to keep on hand specimens of dried oxalated and dialyzed plasma. Dog's blood is oxalated and centrifugalized. The clear plasma is removed and dialyzed in collodion tubes against 0.9 per cent solutions of sodium chloride for twelve to twenty-four hours to remove the oxalate. The plasma is then evaporated in watch crystals, 2 to 5 c.c. to a crystal, in a current of cold air. When dry the plasma may be preserved indefinitely in a desiccator. For use the contents of each crystal are dissolved in 0.9 per cent salt solution, using double the volume of the original plasma. When properly made the dried material dissolves completely, giving a clear solution in which the fibrinogen is in a more normal condition than when obtained by the usual method of repeated fractional precipitation with sodium chloride.

THE RELATION OF METATHROMBIN TO THROMBIN

By F. W. WEYMOUTH

[*From the Physiological Laboratory of the Johns Hopkins University*]

MORAWITZ showed in 1904 that part of the confusion surrounding the question of thrombin has arisen from the fact that there are two inactive forms both of which have been considered as precursors of the ferment. To one of these, found in oxalated plasma and activated by calcium salts, the prothrombin of Pekelharing, he gave the name α-proferment. The second, found in serum subsequent to clotting and not activated by calcium salts but by acids and alkalies in the entire absence of calcium, he called β-proferment; this was identical with the metazym of Fuld.[1] Later[2] he proposed the term metathrombin, a more suitable name in view of the fact that its absence from oxalate plasma and presence in serum only subsequent to thrombin formation shows it to be a modification and not a forerunner of the latter.

Unfortunately all writers have not adhered to this original conception. Mellanby, for instance,[3] after referring to Morawitz's β-proferment uses the term metathrombin for the *active agent* liberated in serum by the action of acids and alkalies, an entirely unwarranted use of the term so clearly defined by Morawitz.

The present paper presents the results of some experimental work, undertaken at the suggestion of Dr. W. H. Howell, with the hope of throwing some light on the relation of metathrombin to thrombin, which according to Morawitz is entirely unknown.

A brief account will first be given of the technic employed, after which the experiments, dealing with serum, thrombin, and metathrombin, respectively, will be considered. The clotting power of thrombin or serum was tested on plasma solutions which were pre-

[1] Hofmeister's Beiträge, 1904, iv, p. 401.

[2] Ergebnisse der Physiologie, 1905, iv, p. 367.

[3] Journal of Physiology, 1909, xxxviii, p. 92.

pared as follows. A dog (or cat) was bled directly into centrifuge tubes containing enough sodium oxalate to give a concentration 0.1 per cent to the mixture. The tubes were at once thoroughly mixed and centrifuged, the plasma drawn off and dialyzed for twenty-four or more hours against a large amount of 0.9 per cent NaCl to remove the oxalate. The plasma was then measured out in watch crystals (usually 3 c.c. to a crystal) and dried rapidly in a current of air at room temperature. Such crystals were kept in a desiccator until used when they were rubbed up in twice their original volume of 0.9 per cent NaCl (6 c.c.) and filtered. This procedure gives clear solutions which clot promptly and firmly upon addition of fresh serum or thrombin and are of very uniform strength. To avoid errors in comparison a particular experiment was always carried out on a single lot of plasma.

In obtaining the specimens of serum the blood was allowed to flow directly into centrifuge tubes and when clotting had taken place the clots were loosened and the whole centrifuged. The clear serum was then drawn off and set aside to be used as needed.

The tests for clotting time were carried out as follows. Varying small amounts of serum (usually 3, 4, and 5 drops) were placed in a series of homeopathic vials and ten drops of a freshly prepared plasma solution added. This was observed usually at five-minute intervals by tilting the vial until the formation of a clot was noted. This method, though it does not give accurate results for times less than two or three minutes, proved very satisfactory in work of the present nature, as there is little trouble from evaporation and the solutions may be observed over periods of twenty-four or more hours.

Non-sterile Sera

The first three experiments were carried out on what may be termed *non-sterile sera*, the serum being collected without aseptic precautions and allowed to stand in uncorked test tubes at room temperature throughout the experiment. Chart No. 1 shows the variations in clotting power of these specimens, the abscissae representing the time in days after the drawing of the blood, and the ordinates the clotting time in hours. The solid lines represent the clotting time in five drops of serum added to ten drops of plasma, and though

similar and consistent data are at hand for three and four drops they have been omitted for clearness. The broken lines represent tests for metathrombin to be considered presently.

The serum in the three experiments shows great variation yet essential agreement in certain features which may be summarized as follows.

1. A period of loss of clotting power, becoming more rapid as the process continues.

   The clotting time of the serum of Experiment No. 1 increased from fifteen minutes to three hours in three days (its further course was not followed). No. 3 when tested at three days gave no clot although the solutions stood for six days. The curve of No. 2 shows a rise almost as rapid though its start is delayed. This is apparently due to the fact that the serum was kept on ice for part of the time between its drawing and the first test. At five days its clotting time had also exceeded three hours. It would appear from this that dog's serum when freely exposed at room temperature (18° to 27° C.) for three to four days has its clotting power so greatly reduced as to escape detection by many of the methods employed; in fact for practical purposes the clotting power may be said to have been lost. The most active serum observed at this time caused clotting in nine times its original period.
2. A second period of marked variations in clotting power. This extended over five days in No. 3 and eighteen days in No. 2; the two experiments show few points of resemblance in this period. Infection is present in this period; it will be convenient to return to this point later.
3. A practically complete return of clotting power. In No. 3 this extended over eight days and reached a point where clotting took place in twenty minutes as against fifteen minutes in the first test. In No. 2 it lasted five days, reaching a clotting time of fifteen minutes as compared with ten minutes in the beginning.
4. A final period of complete loss of clotting power, occurring in eighteen to twenty-nine days.

Although tests were made over a period of thirty-four days in No. 2, and forty-eight days in No. 3, no clots were obtained though the solutions stood twenty-four to forty-eight hours.

Morawitz, who studied the diminution of ferment content in

horse serum, states that, though it varies considerably in different samples, the loss is practically complete in five to six days, the loss at first being more rapid. This corresponds well with the initial period of loss here shown, though to be exact there is clear evidence of thrombic power in samples of dog serum at the end of six days and for a long time afterwards. I am not aware that the serum has been carefully followed over long periods by any previous worker.

Reactivation

Parallel with the tests on the clotting power of these specimens of serum the content of metathrombin was studied and the results are shown by the broken curves in Chart No. 1. The method used was that which Morawitz found most efficient, treatment with an equal volume of $\frac{n}{10}$ NaOH and subsequent neutralization with $\frac{n}{10}$ HCl. The period of incubation was usually twelve minutes. As this process diluted the serum to approximately three times its original volume, a larger amount of the mixture was used, though not enough to offset the dilution. The curve given is based on the clotting time of 6 drops of a mixture of 10 drops serum, 10 drops $\frac{n}{10}$ NaOH and about 10 drops $\frac{n}{10}$ HCl. The process of reactivation does not always give uniform results and has at times failed, due apparently to changes taking place in the solutions of acids and alkalies on standing, yet on the whole the results are consistent and give valuable information.

A more serious question is that of the effect of the alkali on the free thrombin, as the interpretation of the curve depends upon what part of the clotting power of the reactivated samples is due to the persistence of the free thrombin and what to the thrombin liberated from the metathrombin. Morawitz states[4] that the prolonged action of alkalies destroys the liberated thrombin; for instance, the action of $\frac{n}{10}$ NaOH for three hours at 35° C. completely destroyed the clotting power of a solution which if neutralized in less time proved very active. Strong solutions of the thrombin used in later experiments were tested for this point and it was found that even short treatment with $\frac{n}{10}$ NaOH greatly injured the thrombic power and

[4] Hofmeister's Beiträge, 1903, iv, p. 404.

though the results were not always uniform it appears that the amount of thrombin undestroyed by the usual incubation of twelve minutes must be slight.

The features in common shown by the curves representing metathrombin content are: first, an initial loss much slower, however, than that of the serum, and second, a final complete loss corresponding to that of the thrombin. The intervening oscillations are neither consistent in the two experiments nor clearly related to the curves of the serum in the same experiments, sometimes following these and sometimes going in the opposite direction.

Sterilė Sera

It was noted in the preceding experiment that during the period of greatest variation patent signs of putrefaction appeared. (See Fig. 1.) This suggested that more uniform and satisfactory results could be obtained by preventing infection. Accordingly a second series of experiments was carried out in a manner similar to that already described except that sterile cannulae and tubes were used and that the specimens of serum were kept stoppered with cotton except when samples were removed with a sterile pipette. During part of the time cultures were taken to determine the success of these precautions.

Figure 2 represents curves constructed as in the previous case from the results of two experiments. The most striking fact shown is that as long as the solutions are sterile there is no spontaneous return of power either of the thrombin or the metathrombin. The curves which were so variable in the previous experiments have become remarkably uniform. The only exception is shown by the serum of No. 5, which exhibits an increase of power just before its final loss. That this uniformity is due to the sterile condition is clearly demonstrated by the fact that a sample of the serum removed in No. 5 and exposed to the air, became infected and at once showed an increase of clotting power, diverging sharply from the behavior of the still sterile serum.

An equally important fact is that the initial loss is greatly delayed. Up to seven days in No. 6 and to fourteen days in No. 5 the sera

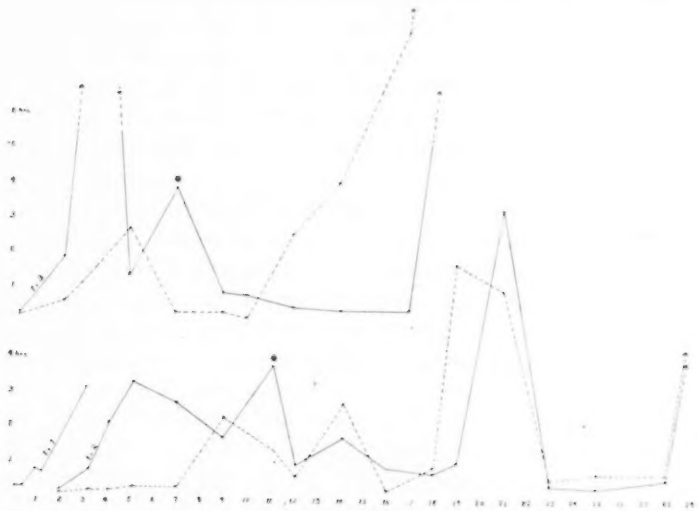

FIGURE 1. Curves from three experiments representing the relative amounts of thrombin and metathrombin in dog's serum (non-sterile) kept for a number of days.

The solid lines give the curves for thrombin, the broken lines the curves for metathrombin. The figures along the abscissa represent days, those along the ordinate give the time in hours for the clotting caused by five drops of serum added to ten drops of the fibrinogen solution (oxalated and dialysed plasma). * Infected.

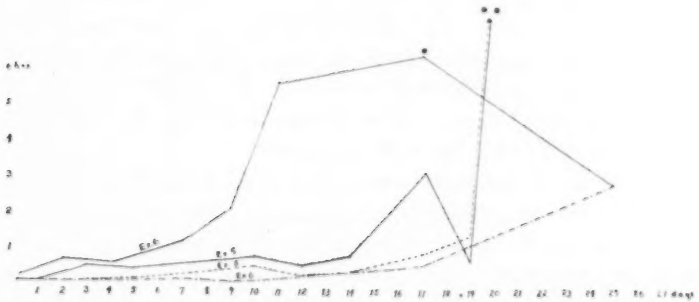

FIGURE 2. Curve of two experiments representing the relative amounts of thrombin and metathrombin in a sterile serum kept for a number of days. * Infected. ** Clotted in seventeen hours on twenty-first day; did not clot again.

are still very active, clotting in seventy and fifty-five minutes respectively. This is even more strikingly the case with the metathrombin, which retained most of its power up to seventeen days in both experiments. Infection, therefore, hastens the initial loss of clotting power, which, however, occurs in its absence, and causes most if not all of the apparently spontaneous returns of thrombic power observed in ordinary serum.

Morawitz mentions various factors which hasten the loss of clotting power.[5] Temperature is important, specimens kept at 35° C. lost power in two and a half days, while those in an ice chest showed power after twelve days. Free exposure to the air also hastened the loss. Since sterile serum in the present work retained its clotting power at a room temperature of 18° to 27° C. for as long a time as that just cited for the serum kept in an ice chest, it is plain that the temperature in itself had little to do with the inactivation. It is clear that the influence of high temperatures and free access of air is to promote infection and bacterial growth rather than directly to affect the serum. Morawitz also states that power is retained longer in a neutral than in an alkaline medium. The present work throws no light upon this fact.

It is interesting to note in connection with the question of loss of thrombic power that suggestively analogous behavior has been found in the case of inorganic ferments. For instance, McIntosh[6] states that on standing, colloidal silver loses some of its power to decompose hydrogen peroxide, due apparently to the fact that on standing the silver precipitates out. The silver solutions were also "reactivated" by alkalies. In reacting some of the silver passes into the hydroxide, which is broken up, liberating free silver. Here both "inactivation" and "reactivation" depend upon definite reactions the counterparts of which we do not yet know for thrombin.

Reduced to their simplest terms and freed from the complication of putrefaction these experiments show that there is a gradual and progressive loss of clotting power in serum so that in the course of one or two weeks the clotting time has increased about fivefold and by another week the serum is completely inactive. Metathrombin runs a similar course though its loss is much less rapid. Though ap-

[5] Hofmeister's Beiträge, 1904, iv, p. 395.

[6] Journal of physical chemistry, 1902, vi, p. 15.

parently a more stable body it does not seem to survive the thrombin for any appreciable time.

The fate of thrombin and metathrombin is not shown by their behavior in serum. According to the method of reactivation, already discussed, metathrombin is present from the outset and in greater amount than at any subsequent time. If the thrombin is transformed into metathrombin during the period of its rapid loss, the amount is too small or its subsequent destruction too rapid to cause an increase in the latter. These experiments, therefore, can throw no light on the origin of metathrombin. The return of clotting power after infection may be due to transformations of one of these substances into the other, but the factors are too complex to be easily analyzed and offer small inducement to investigation in this direction.

Kephaline

Several experiments were carried out in which the effect of adding kephaline solutions[7] to serum was tried. Experiment 15 will serve as an example of the results. Six mixtures were made as follows.

Serum	Kephaline	Water
A 2.5 c.c.	0	1.5
B 2.5 c.c.	0.1	1.4
C 2.5 c.c.	0.25	1.25
D 2.5 c.c.	0.5	1.
E 2.5 c.c.	1.	0.5
F 2.5 c.c.	1.5	0.

After an incubation of fifteen minutes none of the mixtures containing kephaline required more than ten minutes to clot, while *A* clotted only after seventeen hours. About eighteen hours later

[7] These solutions were prepared according to the method described by Howell. This Journal, 1912, xxxi, p. 1.

the mixtures were all less active, showing a very close correspondence to the amount of kephaline added, as indicated by the following table.

Amount of kephaline	Clotting time of 5 drops	Calculated amount of thrombin present on basis of F = 5 d. (Enzyme × clotting time = constant)	Amount of kephaline ÷ calculated amount of thrombin
A 0.	24 hrs.	—	—
B 0.1	155 min.	0.44	0.204
C 0.25	50 min.	1.5	0.167
D 0.5	30 min.	2.5	0.2
E 1.	20 min.	3.75	0.267
F 1.5	15 min.	5.	0.3

At this time, however, the test for metathrombin gave a clotting time of ten minutes for all. On standing all the solutions lost power both in thrombin and metathrombin, though these two maintained about the relation shown in the detailed test just cited. At the end of eight days none of the mixtures clotted under twenty-four hours except *F*, the clotting time of which had reached thirty minutes. In general the other experiments gave similar results.

The following points seem to be shown. The addition of kephaline has no appreciable effect on the amount or rate of disappearance of metathrombin. This would agree with the idea that the metathrombin is formed for the greater part at the time of clotting. The free thrombin present corresponded to the amount of kephaline present, thus agreeing with Howell's conception of its action in neutralizing the antithrombin and so releasing the thrombin. Though it greatly retarded the loss of free thrombin it did not prevent it, even when present in large amounts. This is true of all the experiments tried. This might indicate that the antithrombin-kephaline compound is unstable and slowly breaks up, releasing the antithrombin, or that the thrombin was inactivated by some other substance beside antithrombin.

THROMBIN

The greater number of experiments in the present work were carried out with specimens of thrombin which was made by Howell's chloroform method.[8] At that time he stated (p. 459) that "aqueous solutions of thrombin protected from putrefaction slowly lose their efficiency." The lot from which the solutions here used were made gave very uniform results and these solutions retained their thrombic power for remarkably long times. In all of the experiments controls of thrombin diluted with water were used and in no case under observation was there any loss of power though some of the periods were as great as eighteen days. The power of these thrombin solutions to cause clotting in two and a half to five minutes after standing for two weeks is in marked contrast to that of serum, which under the most favorable conditions will not cause clotting under an hour after similar periods. Some at least of the factors causing loss of power have been removed in preparing the thrombin solutions.

That the thrombin in these solutions is not more stable than that in serum is easily proved, for if some serum is mixed with a thrombin solution the clotting power of the latter is rapidly and completely lost. A considerable number of experiments were carried out in this manner to test the effect upon thrombin of serum treated in various ways. The following may serve as examples of these. It should be remembered that heating plasma to 60° C. coagulates the fibrinogen and destroys the thrombin so that these preparations carry no thrombin into the mixture.

EXPERIMENT 9:

2 c.c. thrombin + 2 c.c. serum heated to 60° C. for 5 minutes

(In this and all other experiments there was a control of thrombin and water which clotted in 2½ or 5 minutes)

Test	Period of incubation	3, 4 and 5 drops + 10 drops plasma sol.	
1	20 min.	4d. weak clot at 70 min.	
2	3 hrs.	5d. clotted in 25 min.	3 and 4d. in 24 hrs.
3	20 hrs.	5d. clotted in 40 min.	4d. in 70', 3 trace in 24 hrs.
4	44 hrs.	5d. clotted in 45 min.	4d. in 6 hrs., 3 no clot in 24 hrs.

[8] This Journal, 1910, xxvi, p. 453.

EXPERIMENT 17:

	Thrombin	Plasma	Water
C	1. c.c.	.2 c.c.	.8 c.c.
D	1. c.c.	.1 c.c.	.9 c.c.

Test	Incubation	
1	20 min.	C clotted at 10 min. D 5d. in 2½ min., 3d. in 10
2	16 hrs.	C no clot in 24 hrs. D very weak clot in 24 hrs.
3	66 hrs.	C and D no clot in 24 hrs.
4	162 hrs.	C and D no clot

EXPERIMENT 18:

C 2 c.c. thrombin + 1 c.c. serum heated to 60° + 1 c.c. H_2O

Test		
1	4½ hrs.	Faint clot in 5½ hrs.
2	41 hrs.	Faint clot in 24 hrs.
3	89 hrs.	No clot
5	7 days	No clot

EXPERIMENT 20:

C 2 c.c. thrombin − 1 c.c. serum heated to 60° − 1 c.c. water

Test		
1	16 min.	Clotted in 10 min.
2	1 hr. 40 min.	Clotted in 115 min.
3	18 hrs.	No clot

From this it appears that serum heated to 60° C. will inactivate twice its volume of an active solution of thrombin in twenty-four hours. In five experiments only two showed a trace of clotting power after standing twenty-four hours. In Experiment 17 the serum inactivated five times its volume in sixteen hours, so that while the control clotted in two and a half minutes the serum-thrombin mixture did not clot in twenty-four hours.

It is not necessary to examine in detail the evidence for all the temperatures tried. Specimens heated to 62° C. show similar behavior though less rapid action. The inactivating agent even survived a temperature of 62° C. for over an hour, and 65° C. weakened but still left it very active. When heated to 70° C. (cat's serum) the mixture after standing eleven days still clotted as promptly as the

control. Pig's serum heated to 80° C. also showed no inactivating power. Unheated serum, as we have seen, has an even more rapid inactivating action than heated sera.

Therefore there exists in fresh serum or oxalated plasma a thermolabile body which acts rapidly upon thrombin converting it to an inactive form. The similarity of action of this body to antithrombin is, of course, obvious. Since this substance even though considerably diluted works so rapidly on a solution of thrombin more active than fresh serum it seems peculiar that serum should, under ordinary circumstances, hold its clotting power for several days. In fact a specimen of serum to which thrombin is added, loses its power more rapidly than it otherwise would. It is difficult to see an explanation for this behavior.

An effort was made to locate the inactivating body by salting out the proteins of the serum. It was found that the first filtrate after half saturation with ammonium sulfate (freed of this salt by dialysis) caused loss of power. This filtrate was further completely saturated to see if the body was an albumin or went out with the albumins. The protein-free filtrate had no inactivating effect but the albumin was unfortunately denatured in the treatment and could not be tested. A second preparation of serum albumin as well as egg and lactalbumin did not show the inactivating agent. Later experiments showed that the body was greatly weakened and finally removed by dialysis, thus explaining the failure to locate it among the proteins.

Calcium

Since the inactivating body is destroyed by heat it cannot be an inorganic salt. Calcium was, however, tried for possible effects. Solutions of $CaCl_2$ of varying strengths, some of them approximating that of the blood, were added to thrombin solutions, but produced no more sign of inactivation during the eleven days of the experiment than did the controls containing distilled water. A second possibility was tested by oxalating serum and dialysing it free of the added oxalate. This calcium-free serum when mixed with thrombin caused as rapid inactivation as ordinary serum. Evidently the presence of calcium does not produce, nor its absence prevent, inactivation.

Hirudin

Since the behavior of the inactivating body toward heat, dialysis, kephaline and other factors clearly identify it with the antithrombin of the blood, hirudin was tested for its effect on thrombin. Much difficulty was experienced in balancing the strengths of the hirudin and the thrombin, and though repeatedly tried it was not found possible to get a mixture in which the original retardation of thrombic power was slight, but which showed on standing the progressive action characteristic of serum. The thrombin was either completely and rapidly inactivated or, if, the initial action was small, this decreased rather than increased.

Kephaline

As kephaline acts only on antithrombin it was not tested on thrombin direct but in conjunction with serum or hirudin. In these cases it retarded the inactivating effect but did not completely prevent it; agreeing thus with the results already given of its action in serum.

Metathrombin

Having considered the fate of thrombin and methrombin in serum and the factors causing inactivation of thrombin solutions, we come to the main purpose of the present work, a study of the characteristics and mode of origin of metathrombin. Morawitz gives the following as characteristic properties of metathrombin.[9] It is not found in oxalate or fluoride plasmas but in all sera whether fresh or old and inactive. It is more resistant toward the factors destroying thrombin, since it is found in as great amount in five-day-old serum as in fresh serum. If treated with acids and alkalies the active thrombin freed rapidly disappears. Apparently all is not freed by the first activation, since it may be reactivated twice, but the freed thrombin does not return to metathrombin as it may not be activated a third time. It is thermolabile, being destroyed (as are thrombin and prothrombin) by half an hour's heating to 60° or 62° C. It is not removed by dialysis and is precipitated out with the globulins by

[9] Hofmeister's Beiträge, 1904, iv, p. 402 et seq.

addition of 30 to 50 per cent ammonium sulfate. It is activated by alcohol as well as acids and alkalies, but prolonged action of the latter destroys it. It is not found in Schmidt's thrombin.

I can confirm many of these points and have little to add. Metathrombin is present in five-day serum in considerable amount but never in the full strength shown at first. It is absent from very old serum. Thrombin prepared by Howell's method shows no metathrombin.

One prevalent misconception must be here corrected. It is stated, for instance,[10] that "the thrombin [destroyed by the action of serum] may be recovered, in part at least, by proper alkali or acid activation." Serum shows metathrombin directly after clotting and it is not necessary to wait until the inactivation of the patent thrombin before attempting activation. In the present work many of the experiments gave misleading results because at first this was not realized. That thrombin is continually going over into metathrombin is quite probable, but if so the amount is too small to affect the much greater initial mass of metathrombin, and even the rapid loss of clotting power of serum in the first three or four days causes *no increase* of metathrombin.

Morawitz [11] considers that since metathrombin is not present in oxalate plasma but is present in great amount after clotting, its origin must be connected with the process of clotting. To determine this point as well as the possible rôle of calcium, he clotted oxalate plasma with Schmidt's thrombin containing oxalate. The serum obtained was inactive because of the great dilution of the thrombin and its absorption by the fibrin. It also contained no *metathrombin*. He therefore concludes that metathrombin is not formed in the process of clotting but arises through the action of calcium on prothrombin as does thrombin. Later [12] he modifies this view slightly, stating that the thrombin for the greater part goes over into metathrombin very quickly after clotting. That metathrombin represents a thrombin-antithrombin compound he considers disproved by the fact that in serum which has lost its antithrombin there is still much metathrombin. On the other hand, since the free thrombin of the serum persists

[10] Rettger, This Journal, xxiv, 1909, p. 430.

[11] Hofmeister's Beiträge, 1904, iv, pp. 405, 411.

[12] Ergebnisse der Physiologie, 1905, iv, p. 371.

so much longer than what is apparently the same thrombin liberated from metathrombin by activation, he concludes that part of the former is bound to antithrombin and is only slowly liberated to disappear almost at once.

In regard to the experiment of clotting oxalate plasma with thrombin, the only point established is that the formation of fibrin from fibrinogen and thrombin does not produce metathrombin. Work of a different character, soon to be detailed, confirms this, and, in fact, such an origin was hardly to be expected. That metathrombin arises from prothrombin through calcium action is not proved, since other hypotheses, for instance the theory here proposed of a thrombin-antithrombin origin, meet equally well the logical requirements which are merely the absence of both thrombin and metathrombin from oxalate plasma and their presence soon after normal clotting, or to be more exact, the opportunity for interaction between prothrombin and calcium in the presence of the other components of the blood. Experiments on this point will be given.

As to the presence of metathrombin in solution from which the antithrombin has disappeared: this, instead of militating against a thrombin-antithrombin compound, is rather what would be expected, since if the antithrombin were bound in any such union it would hardly manifest itself in the ordinary manner.

It has already been stated that thrombin solutions (which contain no metathrombin) are rapidly inactivated by serum. Such solutions were tested not only for thrombin but for metathrombin. Somewhat detailed results of one such experiment may be given.

EXPERIMENT 20:

Five mixtures were made as follows:

			Serum			
				Heated		
	Thrombin	Water	Unheated	60°	65°	70°
A	2 c.c.	2	—	—	—	—
B	2 c.c.	1	1	—	—	—
C	2 c.c.	1	—	1	—	—
D	2 c.c.	1	—	—	1	—
E	2 c.c.	1	—	—	—	1

TEST 1. After 15 minutes' incubation,
5 drops clotted plasma in

A	5 min.
B	20 min.
C	10 min.
D	5 min.
E	2½ min.

TEST 2. After 1 hour and 4 minutes.

5 drops clotted plasma in		6 drops activated clotted in
A	5 min.	—
B	280 min.	10 min.
C	115 min.	115 min.
D	5 min.	115 min.
E	2½ min.	45 (weak)

TEST 3. After 18 hours and 20 minutes.

A	5 min.	65 (weak)
B	24 hrs.	15 (weak)
C	No clot in 24 hrs.	90 (weak)
D	490 min.	No clot in 24 hrs.
E	5 min.	65 min.

TEST 4. After 66 hours.

A	5 min.	20 min.
B	24 hrs.	35 min.
C	No clot in 24 hrs.	160 min.
D	24 hrs.	460 min.
E	10 min.	160 min.

This experiment clearly shows a point already referred to — the destruction of the inactivating body at a temperature between 65° and 70° C. — as *A* and *E* are the only mixtures which retained their thrombic power. The matter which now concerns us, however, is

the presence of metathrombin. In *B*, the first mixture to show loss of power, test 2 reveals a large amount of metathrombin. After this time both thrombin and metathrombin decline in this specimen. In *C*, in which the inactivation is less rapid, the metathrombin appears correspondingly later (test 3). In *A* and *E* there is at no time evidence of any considerable amount of metathrombin, and the readings are due in part at least to incomplete destruction of the large amount of thrombin present by the process of reactivation.

This experiment seems to me to offer in the related loss of thrombin and appearance of metathrombin proof of the transformation of thrombin into metathrombin by an inactivating agent in the serum which we have every reason to identify with antithrombin.

As previously stated, the action of hirudin upon thrombin was studied, but in this case no metathrombin was detected though it was repeatedly sought for. Whether this failure arose from the difficulty of balancing the action of the thrombin and the hirudin — it will be noted that in the experiment just given the whole course of events was far more rapid than in serum, and conceivably thrombin and hirudin may act even more quickly — or from an essential difference between hirudin and the antithrombin of the blood, I cannot say.

If metathrombin arises from the union of thrombin and antithrombin it ought to be possible to obtain a serum free from metathrombin if clotting took place in plasma deprived of its antithrombin. This was attempted by adding kephaline to oxalate plasma and then clotting with $CaCl_2$, but the antithrombin could not be completely removed by this method, and metathrombin, though in reduced amount, was still present. On standing, the antithrombin content of both plasma and serum diminishes, but this did not prove a more satisfactory method. It was found, however, that dialysis removed the antithrombin, and though it weakened the prothrombin so that it was impossible to obtain an absolutely antithrombin-free plasma that would clot on addition of calcium, the antithrombin was very greatly reduced.

In Experiment 27 it was found that after forty-two hours' dialysis, one drop of the plasma (heated to 60° C. to remove the fibrinogen) added to two drops thrombin caused a fibrinogen solution to clot in five minutes, while a similar solution of thrombin and water caused

clotting in two and a half minutes. With fresh plasma the clotting may be retarded from twenty minutes to an hour. Ten c.c. of this plasma were removed and 0.2 c.c. of 2 per cent $CaCl_2$ added, producing clotting in twenty-three minutes. This clot was removed with a stirring rod and the serum tested at once for thrombin and metathrombin. Five drops of the serum caused clotting in five minutes; six drops of a reactivated sample caused no clot in twenty-four hours. A second test, half an hour later, gave similar results except that the reactivated mixture caused a weak clot in five hours which did not become firm on standing over night. Two hours later the metathrombin was increased, though not more than might have been expected from the small amount of antithrombin present. It should be borne in mind that under ordinary circumstances a sample of serum taken half an hour to two hours after clotting will cause clotting in five or ten minutes. Calcium was of course present, but this failed to produce metathrombin, so that Morawitz' conception of an origin from prothrombin and calcium is hardly tenable.

In view of the facts that metathrombin may be produced from a thrombin solution by the addition of antithrombin, and that the absence of antithrombin from an otherwise normal process of clotting prevents the appearance of metathrombin, there can be little doubt that metathrombin is a compound of thrombin and antithrombin. It apparently arises wholly or in great part very soon after clotting from the union of the newly formed thrombin and the antithrombin present in the blood, and then disappears, as does the unchanged thrombin, though much less rapidly.

Summary

The present paper deals with the thrombin content of serum as determined by its clotting power on fibrinogen solutions and the metathrombin content as indicated by the clotting power after "activating" by the addition of weak alkalies and subsequent neutralization with acids. The effect of various substances upon thrombin and on serum was studied in an attempt to find the relation between thrombin and metathrombin.

1. The clotting power of serum (dog) is rapidly lost under ordinary conditions, becoming practically negligible in three or four days.

Following this there is a considerable period (five to eighteen days) of great variation ending with an almost complete return of power and finally after eighteen to twenty-nine days an absolute and lasting loss.

2. What has been said of thrombin is also true of the metathrombin content except that the initial loss is markedly less rapid.

3. If the serum is kept sterile the loss of power is much slower, the solution remaining quite active up to one or two weeks and requiring another week to become entirely inactive. The loss of metathrombin is even slower and in neither is there evidence of spontaneous reactivation. We may conclude that infection hastens the loss of both thrombin and metathrombin and is the cause of the apparently spontaneous return of power observed. Most of the factors which are stated to hasten inactivation act by hastening infection and bacterial growth.

4. Thrombin prepared by the chloroform method of Howell will retain its clotting power absolutely unimpaired for considerable periods (at least eighteen days).

5. The clotting power of this thrombin is rapidly destroyed by a body present in oxalate plasma and in serum. This inactivating body is considered identical with antithrombin from the following characters:

(*a*) It is thermolabile, surviving a temperature of 65° but being destroyed by 70° C.

(*b*) It is weakened and finally destroyed by prolonged dialysis.

(*c*) Its action is retarded by the presence of kephaline.

(*d*) The amount of antithrombin present in serum as indicated by the ordinary test of its retarding effect on a mixture of thrombin and fibrinogen always corresponds to the amount of the inactivating body present.

6. The presence of metathrombin in solutions of thrombin after the inactivation of the latter by antithrombin has been demonstrated at least in certain cases. This seems to make highly probable that metathrombin arises from the interaction of thrombin and antithrombin.

7. The practical absence of metathrombin arising from the clotting of oxalate plasma deprived as far as possible of its antithrombin by dialysis, greatly strengthens the probability that metathrombin is, as indicated above, a thrombin-antithrombin compound.

To recapitulate: it seems highly probable from the present experiments that metathrombin (β-proferment) is a thrombin antithrombin compound, which arises normally in greater part at the time of clotting by union of the newly formed thrombin with the antithrombin present in the blood. It exists parallel with the thrombin, but on account of its greater resistance to destroying influences and probable greater initial amount it persists in greater strength and for a longer time, but finally disappears entirely. Its rôle in ordinary clotting would appear to be negligible; it represents part of the excess of thrombin produced. The theoretical possibility of its origin from protective neutralization of thrombin in circulating blood is not supported by experimental proof, as it has not been found in oxalate or fluoride plasmas.

ON THE ABSORPTION OF WATER BY THE SKIN OF THE FROG

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I. Introduction

THE process of secretion plays so large a rôle among the activities of the animal organism that an understanding of its underlying mechanism would go far to the elucidation of some of the most fundamental problems of physiology. For this reason it would be desirable to examine it in that form which presents the fewest variables. The experiments of Reid [1] and the observations of Overton [2] seemed to indicate that the skin of the frog is able to pass water through itself in opposition to osmotic pressure. If this could be clearly and satisfactorily demonstrated the frog skin could be used for a more thoroughgoing analysis of the process. On first view the results obtained by Reid appear to be convincing, and they seem to have been accepted without question by many physiologists. He experimented by using frog skins as the membranes of osmometers, and compared the rate at which liquid passed through when the outer side was exposed to the lower osmotic pressure with the rate when the inner side was so placed. He found that in healthy living skin the transfer of liquid took place most readily from without inwards, but that this result could be reversed by subjecting the skin to the action of chloroform or by allowing it to become stale. The latter fact, however, the fact that when the skin becomes stale, water passed more readily from within outward, would appear to prove too much. By the use of his very ingenious differential osmometer Reid

[1] Reid: Journal of physiology, 1890, Vol. ii, p. 312.

[2] Overton: Verhanlungen der physikalisch-medicinische Gesellschaft Zu Würzburg, 1904, xxvi, p. 277.

found on comparing the rate of passage of water through the skin three periods:

1. A period in which the quantity of water passing from without inwards is in excess of that passing from within outwards.

2. A period in which the two amounts are practically equal.

3. A period in which the quantity of fluid passing from within outwards is in excess of the quantity passing from without inwards; and the number representing this excess increases with the lapse of time post mortem.

The existence of the first period was taken to prove a vital activity on the part of the cells of the epithelium. The water was supposed to be moved from without inward by a process of secretion on the part of the living skin. By the same logic, however, the existence of the third period proves that the dead skin is also possessed of a secretory power but in the opposite direction, an inference which for obvious reasons no one has ever drawn.

A second set of experiments more to the point was reported by Reid [3] wherein the same liquid was placed in both sides of the osmometer and it was then found that liquid was transferred in the direction from without inward. The liquid used was "normal saline" supposedly isosmotic with the tissues. The concentration is not stated, but at that time normal salt was commonly taken to mean 0.6 to 0.65 per cent NaCl. As we shall see later the concentration is a matter of the utmost importance.

Very extensive experiments on the osmotic transfer of water through the skin of the frog were made by Overton.[4] When the frog was submerged in water large quantities of liquid were taken up, and accumulated in the body in case the discharge of urine was prevented. Even in 0.65 per cent NaCl solution, supposedly isotonic with the tissues, some liquid was still taken up.

Snyder [5] reported experiments on the temperature coefficient of absorption made in the following way: Frog skins were removed without tearing and were tied so as to form little sacs. These sacs were filled with Ringer's fluid, placed in Ringer's fluid and kept at constant temperatures. From the change in quantity of contents the inference was drawn that the process of absorption is a chemical

[3] Reid: British medical journal, 1892 (Feb. 13.) [4] *Loc. cit.*

[5] Snyder: Zentralblatt für Physiologie, 1908, xxii, p. 236.

one. As Snyder does not state the concentration it is to be presumed that he used the original solution of Ringer.

II. Experiments

My first experiments were made to determine approximately the magnitude of the water absorption by the detached skin. It is well known that if a frog's leg is ligatured so tightly as to prevent fluid exchange with the rest of the body a relatively enormous quantity of water is taken up by the leg. While some of this water is later absorbed by the muscles and other tissues and produces pathological changes in them, a large part of the excess of liquid remains free in the interstices and lymph spaces of the limb. Does the detached skin take up water in a similar way? The following experiments will answer.

The skin of the leg was removed with as little violence as possible, any adherent blood gently wiped off, the skin turned right side out and ligatured at both ends. The closed sac thus formed was dipped into distilled water, immediately withdrawn and gently pressed between folds of bibulous paper and weighed. It was then placed in distilled water, and, after twenty-four or forty-eight hours, it was weighed again in a similar manner.

Table I is typical as to range of magnitudes and variability:

TABLE I

Increase of Weight of Empty Skin of Frog's Leg in Distilled Water

Experiment	Weight in Grams			Per cent gain	
	At Beginning	24 hrs.	48 hrs.	After 24 hrs.	After 48 hrs.
1	0.72	1.56		116	
2	0.31	0.79		155	
3	0.68	1.70		150	
4	0.34		1.07		185
5	0.28	0.59	0.78	110	178
6	0.43	1.08	1.43	149	232

It is apparent that a relatively enormous quantity of water passes through a frog skin immersed in water. Is this liquid free or is it in part held in some kind of colloidal solution in the tissues of the skin?

The results in Table II were obtained in the same way as in Table I, but at the close of the experiment the sac was cut open, the liquid emptied out, the empty skin pressed gently between folds of bibulous paper and weighed.

TABLE II

Experiment	Weight			Empty Skin	
	Beginning	48 hrs.	Per cent gain	Weight after 48 hrs.	Per cent gain
1	0.62	1.25	100	0.68	10
2	0.74	1.40	101	0.84	13
3	1.07	2.40	124	1.33	24
4	1.00	2.45	125	1.17	7

The actual swelling of the skin is thus seen to have about the same order of magnitude as that of other tissues immersed in distilled water, while the great proportion of the gain in weight is due to free liquid lying within the sac. The free liquid was always found to be more or less tinged with blood when the sac was opened at the end of twenty-four or forty-eight hours. This was true even when the inside surface of the skin had been carefully washed with water or $\frac{m}{8}$ NaCl at the beginning of the experiment. A not negligible amount of blood had been contained in the cutaneous vessels and affected the result. On this account a few experiments were made of the kind illustrated in Table III. The inner surfaces were washed with water and 2 c.c. of distilled water were placed in each sac before it was tied. The skins were then weighed and placed in salt solutions of varying concentration. So long as these concentrations were low their effect upon the total quantity of liquid absorbed was little different from that of distilled water, but even a small amount of salt tended to inhibit the injurious action on the skin.

TABLE III

SKINS RINSED INSIDE AND 2 C.C. DISTILLED WATER PLACED IN EACH

Experiment	Solution bathing Skin	Weight			Empty Skin	
		Beginning	48 hrs.	Per cent gain	Weight after 48 hrs.	Per cent gain
1	Distilled H_2O	* 5.15	7.38	* 71	3.41	8.
2	M/100 NaCl	5.01	7.23	70	3.03	0.7
3	M/50 NaCl	4.70	6.62	71	2.70	0.
4	N/25 NaCl	4.80	6.71	68	2.78	− 0.7

* Note that the calculations of per cent gain assume that the 2 c.c. of water placed inside weighed 2 grams The error in this assumption does not exceed the necessary experimental error and may properly be neglected.

In the experiments thus far described the skin was immersed in distilled water, and the presence of any of the tissue fluids in the inner layer of the skin would tend by simple osmotic processes to carry water through. Some experiments were then arranged by means of which the liquids in the sac of skin would be diluted while the liquid outside would have a considerable osmotic pressure. The direction and amount of transfer of liquid was noted and this was compared with the osmotic pressure as inferred from the electrical resistance of the liquid without and within the skin at the end of the experiment. The order of magnitudes is illustrated in the Table IV.

TABLE IV

Experiment	Liquid bathing the skin	Liquid placed inside sac	Weight			Specific Conductivity in reciprocal ohms	
			Beginning	After 48 hrs.	Per cent gain	Of bathing solution	Of liquid in sac
1	Tap water	None	0.97	2.14	120	1.45×10^{-3}	2.14×10^{-3}
2	M/25 NaCl	5 c.c. H_2O	11.56	16.07	70	5.19×10^{-3}	5.96×10^{-3}
3	M/15 NaCl	5 c.c. H_2O	7.71	7.42	− 10	10.26×10^{-3}	9.19×10^{-3}
4	M/10 NaCl	5 c.c. H_2O	7.68	6.50	− 40	13.33×10^{-3}	12.90×10^{-3}

NOTE: In experiments 2, 3 and 4 the per cent gain is calculated after deducting 5 gm. as the weight o the water added.

A comparison of the results in Table IV shows as would be expected that the movement of water is in the direction of the lower electrical resistance, that is towards the higher osmotic pressure. It would have been better to have made determinations of the freezing point of the liquids, but the apparatus at hand was not suited to the small amounts of liquids obtainable in some of the experiments. It is perfectly evident, however, that any error due to the presence of non-electrolytes, suspended colloids and the like, would be in the sense of increased resistance, and hence would give appearances favoring the idea of vital activity as opposed to osmotic transfer.

A few experiments were arranged with skins filled with the same liquid as that in which they were immersed. The liquid used was Loeb's solution composed of $\frac{m}{2}$ NaCl 100 parts, $\frac{m}{2}$ $CaCl_2$ 2 parts, $\frac{m}{2}$ KCl 2 parts. Ten cubic centimetres of the solution were placed in each skin and the skin was immersed in fifty cubic centimetres of the same solution. It should be pointed out that these experiments are far from being so crucial as they would seem. Notwithstanding the fact that the skins were rinsed inside and out in the solution, before they were filled and tied, they nevertheless carried with them a considerable quantity of water in the mucous layer on the one side, and of blood and lymph on the other. The attempt to meet this objection by turning some of the skins wrong side out was not wholly satisfactory and it is planned to continue this part of the work in a new form. The sample experiments shown in Table V will, however, give some idea of the conditions observed:

TABLE V

SKINS IN LOEB'S SOLUTION; 10 C.C. OF THE SAME SOLUTION INSIDE EACH SKIN

Experiment	Side out	Weight			Specific Conductivity	
		Beginning	After 24 hrs.	Per cent gain	Of bathing solution	Of liquid in sac
1	Right	13.59	14.01	3.1	13.50×10^{-3}	13.82×10^{-3}
	Wrong	13.81	13.45	− 2.6	13.46×10^{-3}	12.57×10^{-3}
2	Right	12.93	13.09	1.2	12.62×10^{-3}	12.24×10^{-3}
	Wrong	11.52	11.10	− 3.6		
3	Right	14.75	14.93	1.2	12.57×10^{-3}	12.57×10^{-3}
	Wrong	12.84	12.66	− 0.6		

It is probable that a careful removal of excess of liquid from the skins used in experiments 2 and 3 of Table V would have changed the result of the conductivity experiments considerably, but it is a question how much of this can be done without injury to the skin. Experiment 3 would seem to indicate that transfer of liquid can take place without difference of osmotic pressure; but it is possible that such a difference had existed and that in this case equilibrium was established within twenty-four hours.

III. Discussion of Results

It will be seen that while the above experiments show an enormous tendency of the frog skin to transport water by osmosis, the skin appears to be highly impermeable to inorganic salts. Some of the experiments, however, seem to show an ability to transfer water from one side to the other when the same osmotic pressure exists on both sides. The amount of such transfer is strikingly small as compared with that obviously due to osmotic pressure, and also as compared to the work done by secreting organs like the kidneys or the salivary glands. This difference raises the question whether the inference is justified that any transfer of water taking place through the skin is due to vital activity of the living cells. The experiments of Reid [6] commonly cited in support of such a view are not very convincing. He found, for example, that the osmotic effect of a 5 per cent solution of glucose in normal saline in one side of the osmometer, against a normal saline solution on the other, was more effective when the inner side of the skin was turned towards the glucose than when in the reverse position. In the clearest set of experiments reported by him [7] the amount of liquid passed through in twenty-four hours in the one direction was 174.093 cubic millimetres in the other 79.035 cubic millimetres. The difference of the two is 95 cubic millimetres. According to the secretion theory this difference is due to an acceleration in the one direction and a retardation in the other and is brought about by the vital activity of the cells. The actual secretion would then be approximately equal to one half the difference or 47.5 cubic millimetres. The area

[6] *Loc. cit.* [7] *Loc. cit.*, p. 326.

of skin exposed in the osmometer used by Reid was 95 square millimetres and the total effect was equal to the transfer of a layer of liquid one half a millimetre deep over the entire surface of the skin, an amount which could be very well due to the physical differences of the two sides of the skin. On the one side is a layer of tissues infiltrated with blood and lymph; on the other an adherent layer of watery mucous secretion. This arrangement would favor movement of water by osmosis from the outer to the inner side of the skin. When liquids of equal osmotic pressure were placed on the two sides of the skin, movement of water from outside to inside would proceed until the salt concentration of the mucous layer became equal to that of the surrounding liquid. The irreciprocity of permeability to sodium ions described by Bayliss [8] is harder to understand but may be accounted for on analogous grounds. Moreover, Starling [9] has given theoretical considerations to prove that under certain conditions substances may actually pass through a membrane against osmotic pressure without the interference of vital activity. It is highly desirable that the conditions which he assumes should be worked out experimentally.

It has also been shown by Reid that the rate of transfer of water through the frog skin is affected by the addition of chloroform and of alcohol to the liquids in the osmometer, and the inference has been drawn that this is due to an effect of these agents upon the protoplasmic activities of the epithelial cells. In view of the slender evidence for the apparently slight amount of secretion by the skin, it would seem much more reasonable to suppose that these agents bring about some change in the physical state of the epithelium itself or in the adherent layers of material.

It has been pointed out already in the introductory part of this article that a difference in rate according to direction of transfer of liquid through the skin does not of itself prove a vital activity as the cause of the difference, for such a difference exists also in dead skin. In the latter the direction of easier transfer is from within outwards. The differences dealt with are differences in *rate* of transfer. It is noteworthy that the emphasis in most of the work on osmosis has been laid upon *equilibrium* rather than on *rate*, while

[8] Bayliss, Zeitschrift für Biochemie, 1908, xi, p. 226.

[9] STARLING: The fluids of the body, London, 1909.

diffusion has been studied from the standpoint of movement. When we have an adequate knowledge of the dynamics as well as of the statics of osmosis it will be easier to understand the apparent anomalies of the movement of water through the frog's skin without invoking vital activity as a means of explanation.

Summary

1. It has been shown that an empty frog skin immersed in water takes up a relatively enormous quantity of water.

2. The taking up of large quantities of water depends upon the permeability of the frog skin to water and its relative impermeability to inorganic salts.

3. A skin exposed to liquids of equal osmotic pressure on both sides may still transfer water through itself from without inwards, but the amount is relatively small and is probably due to physical differences of the layers of liquid carried by the skin on and within its opposite surfaces.

4. The assumption of a vital activity on the part of the frog skin in transferring water through itself is shown to be unnecessary.